Terrestrial support of pelagic consumers: patterns and variability revealed by a multilake study

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SUMMARY

1. Lake food webs can be supported by primary production from within the lake, organic matter imported from the catchment or some mixture of these two sources. Generalisations about food-web subsidies to lake ecosystems are often based on data from only a few ecosystems and therefore do not consider the potential variability of subsidies among diverse ecosystems in a landscape.

2. We measured the variation among lake ecosystems in terrestrial (allochthonous) utilisation by pelagic consumers and developed models to describe the variability. Stable isotope ratios of hydrogen and carbon were measured for *Chaoborus* spp. and crustacean zooplankton taxa in 40 lakes to quantify consumer allochthonous resource use (allochthony).

3. The median fraction of consumer allochthony estimated using a two-source Bayesian mixing model varied between 4 and 82% (mean among all lakes = 36%) for *Chaoborus* sp. among lakes and between 1 and 76% in a more limited sample of crustacean zooplankton consumers. The degree of allochthonous resource use increased linearly with the availability of allochthonous resources.

4. Terrestrial support of *Chaoborus* was correlated (using best fitting relationships) with covariates for lake organic matter sources including dissolved inorganic carbon, total phosphorus, chlorophyll α, colour and catchment area. However, the most parsimonious model was an inverse relationship between lake surface area and consumer allochthony, indicating that allochthonous subsidies are more important in smaller than larger systems. Given the preponderance of small waterbodies, allochthonous subsidies are important in a large number of lake ecosystems.

Keywords: food webs, lakes, pelagic, stable isotopes, zooplankton

Introduction

Aquatic ecosystems are intimately connected to their surrounding terrestrial catchments (Polis, Anderson & Holt, 1997). The magnitude and importance of terrestrial (allochthonous) subsidies to aquatic food webs are well documented for stream ecosystems (Vannote *et al.*, 1980) where subsidies can stabilise population dynamics and predator–prey interactions and influence carbon flow through food webs (Schindler *et al.*, 1997; Loreau & Holt, 2004; Tank *et al.*, 2010). However, most lake studies assume that food-web dynamics are largely independent of allochthonous inputs even though allochthonous material dominates organic matter pools in many lake regions where eutrophication has not occurred (Jansson *et al.*, 2000; Wilkinson, Pace & Cole, 2013). There is a developing perspective that allochthonous resources are important for aquatic consumers in many non-eutrophic systems (Marcarelli *et al.*, 2011), but further investigation is needed to quantify how and why aquatic food webs vary in their dependence on terrestrial resources.

Zooplankton can assimilate allochthonous organic matter either through the consumption of bacteria that are utilising allochthonous dissolved organic matter (DOM; Jones *et al.*, 1999; Jansson *et al.*, 2007) or through

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the ingestion of allochthonous particulate organic matter (POM; Cole *et al.*, 2006; Brett *et al.*, 2009). Although the nutritional quality of allochthonous DOM is generally poorer than phytoplankton DOM (autochthonous; Wetzel, 1995), there is clear evidence that it is utilised as an energy source by bacteria (Kritzberg *et al.*, 2006; Berggren *et al.*, 2010; Karlsson *et al.*, 2012). DOM can also contribute to the POM pool through flocculation (von Wachenfeldt & Tranvik, 2008), and benthic–pelagic coupling in lakes allows for the mobilisation of benthic allochthonous POM (Bartels *et al.*, 2012). Differences in the allochthony of zooplankton taxa due to feeding strategy differences have been documented in a number of systems (Karlsson *et al.*, 2004; Pulido-Villena, Reche & Morales-Baquero, 2005). In particular, cladocerans consume bacterial carbon and possibly detrital carbon, generally increasing their allochthony relative to other more selective crustaceous zooplankton such as copepods (Karlsson *et al.*, 2007, 2012; Cole & Solomon, 2012; Faithfull *et al.*, 2012). In addition to allochthonous inputs, vertical spatial heterogeneity in aquatic primary production may be important for migrating consumers (Matthews & Mazumder, 2006; Francis *et al.*, 2011; Batt *et al.*, 2012). Exploitation of deep-water (metalimnetic) resources could be a key pathway for autochthonous support of zooplankton consumers, particularly in deep, clear-water systems (Francis *et al.*, 2011).

A common tool for studying allochthonous subsidies is the stable isotope ratios of ^{13}C and ^{15}N in organic matter and consumers. Using this method, evidence of variable and, in some cases substantial (>50%), allochthonous support has been found for zooplankton in subarctic (Meili *et al.*, 1996; Karlsson *et al.*, 2003; Kankaala *et al.*, 2010), alpine (Pulido-Villena *et al.*, 2005) and temperate lakes (del Giorgio & France, 1996; Grey, Jones & Sleep, 2001; Mohamed & Taylor, 2009). However, there is large natural variation and often overlap in the $\delta^{13}C$ of terrestrial and aquatic resources (Gu, Schelske & Waters, 2011), making quantitative estimates difficult in many systems. Experimentally enriching the inorganic carbon pool with ^{13}C increases the difference between aquatic and terrestrial resources (Cole *et al.*, 2002; Pace *et al.*, 2004, 2007; Carpenter *et al.*, 2005; Taipale *et al.*, 2008). However, this method is costly, making it difficult to apply to a large number of systems. Hydrogen stable isotope ratios (δ^2H) have also been used to quantify substantial allochthonous resource utilisation by zooplankton consumers (e.g. Caraco *et al.*, 2010; Cole *et al.*, 2011; Solomon *et al.*, 2011; Karlsson *et al.*, 2012). Using the ratio of deuterium to protium is advantageous because there is a large separation between terrestrial

and aquatic material and well-constrained estimates of resource isotope ratios (Doucett *et al.*, 2007).

The majority of studies identifying allochthonous resources as an important subsidy for zooplankton are focused on one or a few systems. Very few studies have examined a larger number of lakes (note, however, studies by Karlsson *et al.*, 2003; Mohamed & Taylor, 2009; Francis *et al.*, 2011), so there is not a comprehensive model to explain the variation in allochthonous resource utilisation. Patterns in ecosystem size, trophic status (Grey, Jones & Sleep, 2000) and allochthonous resource utilisation have emerged from previous studies, indicating that allochthony is greater in small relative to large lakes (Zigah *et al.*, 2012) and also greater in nutrient-poor relative to nutrient-rich lakes (Pace *et al.*, 2007; Batt *et al.*, 2012). Patterns in carbon mobilisation and allochthonous resource use have also been identified, indicating that lakes with high bacterial and low primary production have high consumer allochthony (Karlsson *et al.*, 2003; Ask *et al.*, 2009; Solomon *et al.*, 2011). The generality of these patterns needs evaluation in a large number of diverse systems to more strongly test relationships between ecosystem characteristics and allochthonous resource dependence.

The goal of this study is to examine the variability in allochthonous resource utilisation by pelagic consumers in lakes to develop a more comprehensive understanding of the importance of allochthonous subsidies to the pelagic food web. The pelagic predator *Chaoborus* spp. was sampled for stable isotope analysis in 40 lakes encompassing a gradient of allochthonous and autochthonous resource composition (POM and DOM; Wilkinson *et al.*, 2013) to determine the pattern of reliance on allochthonous resources. Crustacean zooplankton were also collected in a representative subset of the lakes ($n = 18$) within which it was feasible to sample common taxa. Metalimnetic POM samples were collected in lakes with the potential for substantial metalimnetic autochthonous resource pools indicated by a deep dissolved oxygen maximum ($n = 15$). We also collected data on the morphological, chemical and biological characteristics of each lake to test the significance of lake size and trophic state in determining consumer allochthony.

Methods

Site description

The 40 lakes used in this survey are located in the Northern Highlands Lake District of northern Wisconsin and the Upper Peninsula of Michigan, U.S.A. The region

is largely forested, with little human development (Magnuson *et al.*, 2006). The lakes were sampled once during a 65-day period in the summer of 2011, and therefore, the samples provide point estimates of the resource pool and consumer allochthony. All of the lakes were thermally stratified during sampling. The lakes represent a large gradient in water colour (absorbance at 440 nm), chlorophyll α concentrations and their ratio, a potentially important indicator of allochthony (Pace *et al.*, 2007; Batt *et al.*, 2012). Information on the morphological, biological and chemical properties of each lake can be found in Wilkinson *et al.* (2013).

Sample collection

Particulate organic matter and DOM samples were taken at a depth of 0.5 m below the surface over the deepest point in the lake. In 15 of the 40 lakes, a metalimnetic oxygen maximum was detected. The oxygen maximum always occurred below the thermocline (operationally defined as the half metre over which there is the greatest change in temperature in the water column). In these lakes, metalimnetic POM and DOM samples were taken at the depth of the oxygen maximum (potential indication of metalimnetic primary production) determined at the time of sampling using an optical dissolved oxygen probe (YSI Incorporated, Yellow Springs, OH). Water samples for hydrogen stable isotope analysis were also taken at 0.5 m and at the oxygen maximum, where appropriate, and stored at 4°C in glass scintillation vials with no aeration. Water samples for dissolved inorganic carbon (DIC) stable isotope analysis from which calculations of carbon dioxide isotopes were made (Zhang, Quay & Wilbur, 1995) were taken at the same depths, stored in gas-tight amber borosilicate vials and preserved with sodium azide.

Chaoborus and zooplankton were collected at or near the deepest point of the lake. *Chaoborus* were chosen as the representative constituent of the pelagic food web because as predators they integrate the varying allochthony signals of their zooplankton prey and are conspicuously present in many lake ecosystems. Larger, late-instar *Chaoborus* spp. were targeted for sampling to avoid any ontogenetic diet changes (Persaud & Dillon, 2009). Zooplankton samples were collected during the day via vertical net tows using a conical net with a mesh size of 80 μm. The maximum depth of each tow was two-thirds of the total depth of the water column at the sampling location. Enough organisms were collected for a bulk zooplankton sample of mixed species composition for isotope analysis for each lake. In 18 of the lakes,

samples of individual zooplankton taxa were separated by hand under a dissecting microscope for isotope analysis. The single taxon samples were considered separately in the mixing model analysis. This subset of lakes was chosen for individual zooplankton taxa analysis based on the ease of separating individual species from the bulk sample (largely based on size), yet maintaining representation of the colour:chlorophyll α gradient in the 40 lakes. For presentation of the results, zooplankton were grouped into two orders, Cladocera and Copepoda, to compare similar taxa among lakes and detect potential differences in allochthonous resources utilisation related to feeding strategy (Barnett, Finlay & Beisner, 2007). *Chaoborus* were collected at night, at least 1 h after sunset, to accommodate any movement from diel vertical or horizontal migration. *Chaoborus* were collected via oblique and vertical towing using a conical net with a mesh size of 153 μm. Organisms were kept in lake water in a cooler on ice and processed within 12 h of collection.

Chaoborus were separated from the net hauls and inspected under a dissecting microscope to ensure that there was no contamination from particles or other organisms. Bulk zooplankton samples were also inspected under a dissecting microscope to identify the dominant zooplankton taxa in each sample and to ensure that there was little contamination from algae, detritus or *Chaoborus*. All organic matter samples were dried at 60°C for at least 48 h, ground to a fine powder and stored in a desiccator in borosilicate vials until they were packaged for stable isotope analysis.

Samples for chlorophyll α concentration, dissolved organic carbon (DOC) and DIC concentration and water colour were taken at 0.5 m and at the depth of the oxygen maximum, where appropriate. Samples for pH and total phosphorus (TP) were taken only at 0.5 m. A detailed description of the collection, processing and analytical methods can be found in Wilkinson *et al.* (2013). DIC concentration was measured simultaneously with ^{13}C-DIC.

Stable isotope analysis

All isotope samples were analysed by the Colorado Plateau Stable Isotope Laboratory (CPSIL, Northern Arizona University, Arizona). Following the procedure used in Doucett *et al.* (2007) for obtaining the isotope ratio of the non-exchangeable H fraction, solid samples for δ^2H analysis were corrected for exchange with ambient water vapour using the bench top equilibration method including a series of internal standards includ-

ing algae. Exchangeable H is typically a low proportion of total H, and unless sample and reference materials have large differences in the relative fractions of exchangeable H, the effect of exchangeable hydrogen on hydrogen isotope ratio measurements should be low (Chesson *et al.*, 2009). Organic matter samples were pyrolysed and analysed using a Thermo-Finnigan TC/EA and Delta^{PLUS}-XL (Thermo Electron Corporation, Bremen, Germany). Cavity ring down laser spectroscopy was used to analyse water samples for δ^2H using Los Gatos Research Off-Axis Integrated Cavity Output coupled to a CTC LC-PAL liquid autosampler. Samples for δ^{13}C were analysed following standard procedures of the laboratory. All values are reported in per mil notation (‰) and are in relation to the international standards of Vienna PeeDee Belemnite (δ^{13}C) and Vienna Mean Standard Oceanic Water (δ^2H).

Bayesian mixing model

A two-source (epilimnetic phytoplankton and allochthonous material) hydrogen isotope Bayesian mixing model was used to determine the allochthonous contribution to consumer biomass in each lake. Additionally, a three-source (epilimnetic phytoplankton, metalimnetic phytoplankton and allochthonous material), two-isotope (δ^{13}C and δ^2H) Bayesian mixing model was also used in the 15 lakes in which there was a detectable metalimnetic O_2 maximum. The mixing models use the previously published methods of Solomon *et al.* (2011) and Batt *et al.* (2012). The model takes into account both the uncertainty in the consumer and the uncertainty in the end members. We provide a detailed account of the computations for *Chaoborus* in Appendix S1.

The allochthonous hydrogen and carbon stable isotope end members (δ^2H$_{Terr}$, mean ±SD = −129.5 ± 15.2‰; δ^{13}C$_{Terr}$ = −29.2 ± 1.5‰) were determined from 81 leaf samples from trees in the catchments of lakes used in this study (Solomon *et al.*, 2011). The δ^2H phytoplankton end members (epilimnetic and metalimnetic) were estimated for each lake and each depth using the measured isotopic composition of lake water and the isotopic discrimination factor.

$$\delta^2\text{H}_{\text{Phytoplankton}} = \delta^2\text{H}_2\text{O} - \varepsilon_\text{H} \tag{1}$$

In eq. 1, δ^2H$_2$O is the hydrogen isotope ratio of the lake water in which the phytoplankton is growing and ε_H is the discrimination factor of phytoplankton against δ^2H$_2$O (see Table S1). The mean and standard deviation of ε_H (160.9 ± 17.0‰) were calculated from pure algal net tows in 10 of the lakes (where pure algal net tows

were possible) used in this study that were visited during an algae bloom and the values reported from the regrowth experiments in Solomon *et al.* (2011) ($n = 4$). Algal samples were inspected under a dissecting microscope for purity and prepared and analysed in the same manner as the POM samples. Similarly, the δ^{13}C phytoplankton end member in each lake with a metalimnetic O_2 maximum and at each depth was estimated using the following equation

$$\delta^{13}\text{C}_{\text{Phytoplankton}} = \delta^{13}\text{CO}_2 - \varepsilon_\text{C} \tag{2}$$

in which δ^{13}CO$_2$ is the carbon isotope ratio of the aqueous carbon dioxide in the lake and ε_C is the discrimination of phytoplankton against δ^{13}CO$_2$. The mean and standard deviation of ε_C (-14.49 and 2.6, respectively) were calculated using the same 10 algal net tow samples analysed for δ^{13}C and the corresponding values of δ^{13}CO$_2$.

Consumer δ^2H in each lake was corrected for the isotopic contribution of dietary water. Dietary water contribution was calculated for consumers in each lake, as described in Appendix S1. The dietary water contribution is compounded at each trophic step. We assumed trophic position = 1 for all cladocerans and calanoids and trophic position = 2 for *Chaoborus* and *Mesocyclops*. Consumer δ^2H was corrected for the dietary water contribution, as described in Solomon *et al.* (2009) and Appendix S1. These estimates were employed in the analyses of dietary source contributions. The determination of source proportions using the two-source mixing model employed the mixing equation

$$\delta^2\text{H}_{\text{Corrected } Chaoborus} = (\phi_{\text{Terr}} * \delta^2\text{H}_{\text{Terr}}) + (\phi_{\text{Phyto}} * \delta^2\text{H}_{\text{Phyto}}) \tag{3}$$

subject to the constraint $\phi_{\text{Terr}} + \phi_{\text{Phyto}} = 1$ in each lake. Here, δ^2H$_{\text{Corrected } Chaoborus}$ is the δ^2H of *Chaoborus* corrected for dietary water, ϕ_{Terr} is the allochthonous fraction of the consumer biomass, and ϕ_{Phyto} is the phytoplankton fraction of the consumer biomass. Analogous equations were used for other consumers. For further details on computing the source portions, see Appendix S1.

To determine whether metalimnetic phytoplankton was an important autochthonous resource, a three-source, two-isotope (δ^2H and δ^{13}C) Bayesian mixing model was used for *Chaoborus* in the 15 lakes with a metalimnetic oxygen maximum. The three potential source fractions were terrestrial material (ϕ_{Terr}), epilimnion phytoplankton (ϕ_{Epi}) and metalimnetic phytoplankton (ϕ_{Meta}). The δ^2H$_{\text{Corrected } Chaoborus}$, δ^{13}C$_{Chaoborus}$ and the end member distributions described above were used as informative priors in the mixing model

$$\delta^2 H_{\text{Corrected Chaoborus}} = (\phi_{\text{Terr}} * \delta^2 H_{\text{Terr}}) + (\phi_{\text{Epi}} * \delta^2 H_{\text{Epi}})$$
$$+ (\phi_{\text{Meta}} * \delta^2 H_{\text{Meta}})$$
$$\delta^{13} C_{\text{Chaoborus}} = (\phi_{\text{Terr}} * \delta^{13} C_{\text{Terr}}) + (\phi_{\text{Epi}} * \delta^{13} C_{\text{Epi}})$$
$$+ (\phi_{\text{Meta}} * \delta^{13} C_{\text{Meta}})$$
$$(4)$$

subject to the constraint $\phi_{\text{Terr}} + \phi_{\text{Epi}} + \phi_{\text{Meta}} = 1$. For further details on computing the source portions, see Appendix S1.

Regression analysis

The *Chaoborus* and zooplankton taxa $\delta^{13}C$ and δ^2H data corrected for dietary water were evaluated using a graphical, gradient-based mixing model and linear regression (Mohamed & Taylor, 2009). This approach uses the natural variation among lakes in $\delta^{13}CO_2$ and δ^2H_2O (the C and H substrates for autochthonous photosynthesis) as well as the average phytoplankton discrimination against both of these inorganic resources to evaluate the linear relationship between consumer δ^2H and $\delta^{13}C$ and the inorganic isotope ratios. If the consumers in lakes only utilised allochthonous material, the consumer δ^2H and $\delta^{13}C$ values would align with the terrestrial end member plotted on the figure (mean allochthonous value being the *y*-intercept, the slope would be zero). Similarly, if the consumer biomass is dominated by autochthonous material, the δ^2H and $\delta^{13}C$ data would vary systematically with δ^2H_2O and $\delta^{13}CO_2$. If consumer biomass was only derived from autochthonous sources, the δ^2H and $\delta^{13}C$ data would only deviate from a perfect regression due to variability in photosynthetic discrimination.

Multiple linear regression analysis was performed to explain the variation in consumer allochthony (*Chaoborus* and zooplankton ϕ_{Terr}, from Eq. 3). The analysis was performed using all combinations of independent variables related to the morphological, biological and chemical properties of each lake. Models were ranked based on Akaike information criteria corrected for small sample size (AIC$_C$; Burnham & Anderson, 2002). Relative to the most likely AIC$_C$ models, those models with a ΔAIC$_C > 4$ were discarded as they were highly unlikely models given the data.

Results

Allochthonous resource use

All of the *Chaoborus* δ^2H values, corrected for dietary water contribution, were within the bounds of the allo-

chthonous and autochthonous end members (Figure 1a). The *Chaoborus* $\delta^{13}C$ values were scattered and did not completely fall within the bounds of the terrestrial and phytoplankton end members (Figure 2a). The *Chaoborus* $\delta^{13}C$ values that fell outside the end member boundaries are probably the result of variability in this measure in relation to the small difference in the end members and the uncertainty associated with equation 2 (discussed below). Using the graphical, gradient-based linear regression approach of Mohamed & Taylor (2009), the slope of a linear regression between $\delta^2H_{Chaoborus}$ and δ^2H_2O was non-significant (Figure 1a). The same was true for the slope of cladoceran and copepod δ^2H and δ^2H_2O (Figure 1c). The same linear regressions performed using consumer $\delta^{13}C$ and $\delta^{13}CO_2$ were also all non-significant (Figure 2). The lack of a significant regression demonstrates that support entirely from autochthonous sources for the population of lakes is highly unlikely (Mohamed & Taylor, 2009).

Medians of the posterior distributions of the source proportions (ϕ_{Terr}) indicate varied degrees of allochthonous support of *Chaoborus* (Figure 1b). The range of *Chaoborus* ϕ_{Terr} estimated from the two-source mixing model was 0.04–0.82 among lakes (Figure 1b). Systems with the highest allochthony (>60%) were small, dark-water lakes, and systems with the lowest allochthony (<10%) were large, clear-water lakes. The median *Chaoborus* ϕ_{Terr} in the entire set of survey lakes was 0.35. For the 15 lakes with potentially distinct metalimnetic resource pools, the range of *Chaoborus* ϕ_{Terr} estimated from the two-source mixing model was 0.04–0.64. The range of *Chaoborus* ϕ_{Terr} for the same 15 lakes estimated using the three-source mixing model was 0.03–0.61. *Chaoborus* ϕ_{Terr} values from the two-source and three-source models were on average only separated by 0.05 for an individual lake. Thus, inclusion of a third potential resource in the model did not significantly change estimates of *Chaoborus* ϕ_{Terr} generated using the two-source model (*t*-test, *P*-value = 0.75). Median *Chaoborus* ϕ_{Meta} ranged from 0.18 to 0.45, and the median *Chaoborus* ϕ_{Epi} ranged from 0.21 to 0.47 (Figure 3).

The δ^2H of individual zooplankton taxa in the lakes also fell within the bounds of the end members (Figure 1c), whereas not all zooplankton $\delta^{13}C$ values were within the end member bounds (Figure 2b). Again, there was no significant correlation between consumer δ^2H and δ^2H_2O or between consumer $\delta^{13}C$ and $\delta^{13}CO_2$. There were 15 cladoceran samples, representing 4 genera (1 *Bosmina* sample, 10 *Daphnia* samples, 1 *Diaphanosoma* sample and 3 *Holopedium* samples) from 14 lakes. There were 17 copepod
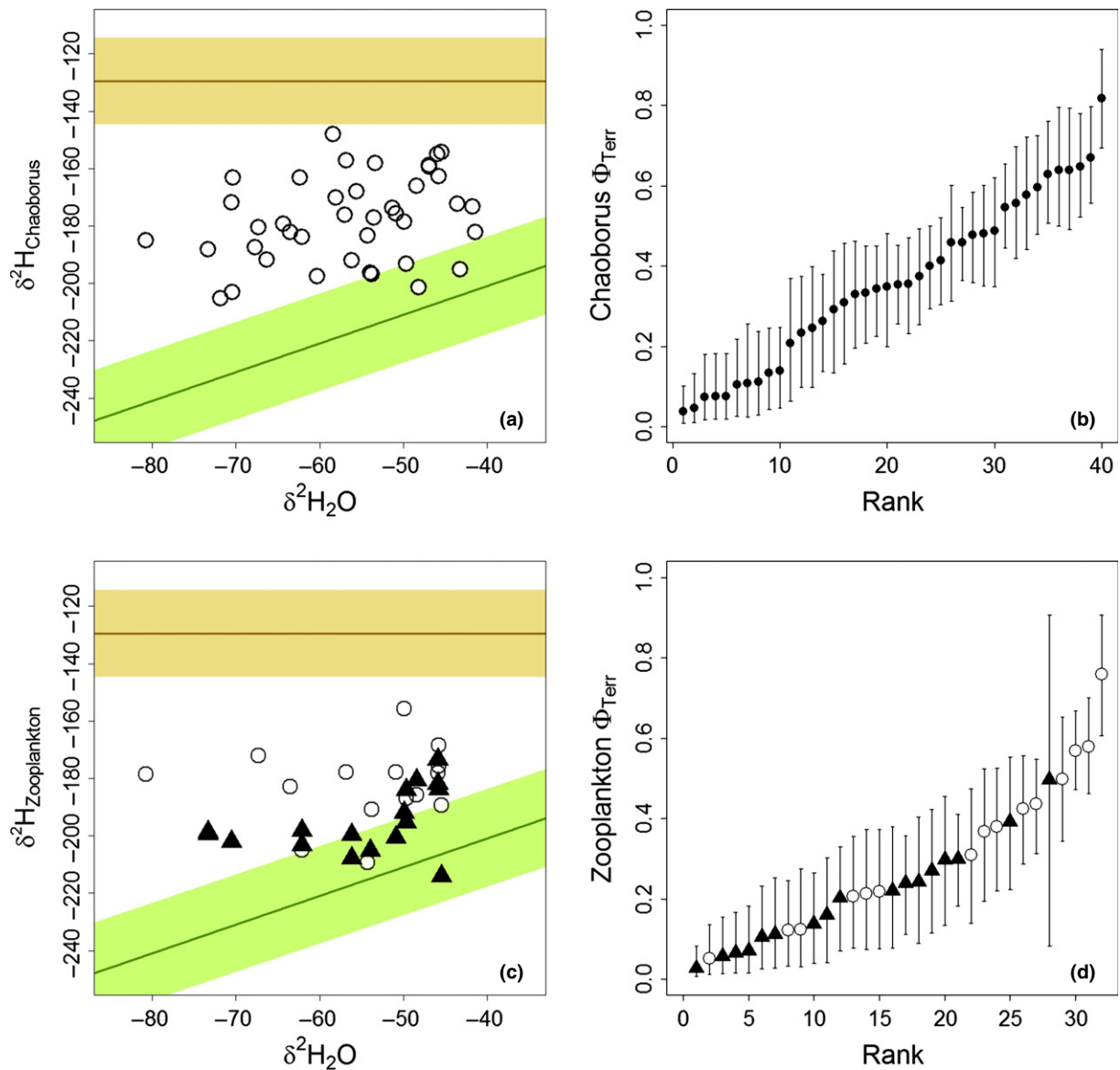
Fig. 1 (a) *Chaoborus* δ^2H in each of the 40 lakes, also showing δ^2H of the allochthonous material (brown line = mean, shaded region = SD of 81 samples, δ^2H not influenced by the δ^2H of the lake water) and of phytoplankton (green line = mean ε_H, shaded region = ε_H SD, phytoplankton δ^2H is dependent on the δ^2H of lake water; see Equation 1). (b) Median of the posterior distribution of *Chaoborus* ϕ_{Terr} with 25 and 75% quartiles (bars) based on the two-source Bayesian mixing model. (c) Cladoceran δ^2H (white circles) and copepod δ^2H (black triangles) in each of the 40 lakes plotted with the end members (same as A). (d) Median of the posterior distribution of cladoceran (circles) and copepod (triangles) ϕ_{Terr} with 25 and 75% quartiles (bars) based on the two-source Bayesian mixing model.

samples, representing 2 taxa (9 calanoid samples and 8 *Mesocyclops* samples) from 12 lakes. The range of cladoceran ϕ_{Terr} was 0.05–0.76, and the range of copepod ϕ_{Terr} was 0.03–0.50 (Figure 1d). The mean cladoceran ϕ_{Terr} was 0.35 ± 0.20 (SD). The mean copepod ϕ_{Terr} was 0.20 ± 0.13 (SD). Cladoceran ϕ_{Terr} was significantly higher among systems than copepod ϕ_{Terr} (*t*-test, *t*-statistic = 2.59, d.f. = 30, *P*-value < 0.01). There was no significant correlation between zooplankton ϕ_{Terr} and *Chaoborus* ϕ_{Terr} from the same lake.

Among lake patterns in allochthony

There were 14 linear models predicting allochthony, containing one or two independent variables with a $\Delta AIC_C < 4$ (Table 1). These models contained some combination of eight variables, four of which were positively correlated with *Chaoborus* ϕ_{Terr} (water colour, colour:TP, colour:Chla, POM ϕTerr) and four of which were negatively correlated with *Chaoborus* ϕ_{Terr} (lake surface area, catchment area, DIC, TP). The most parsimonious model
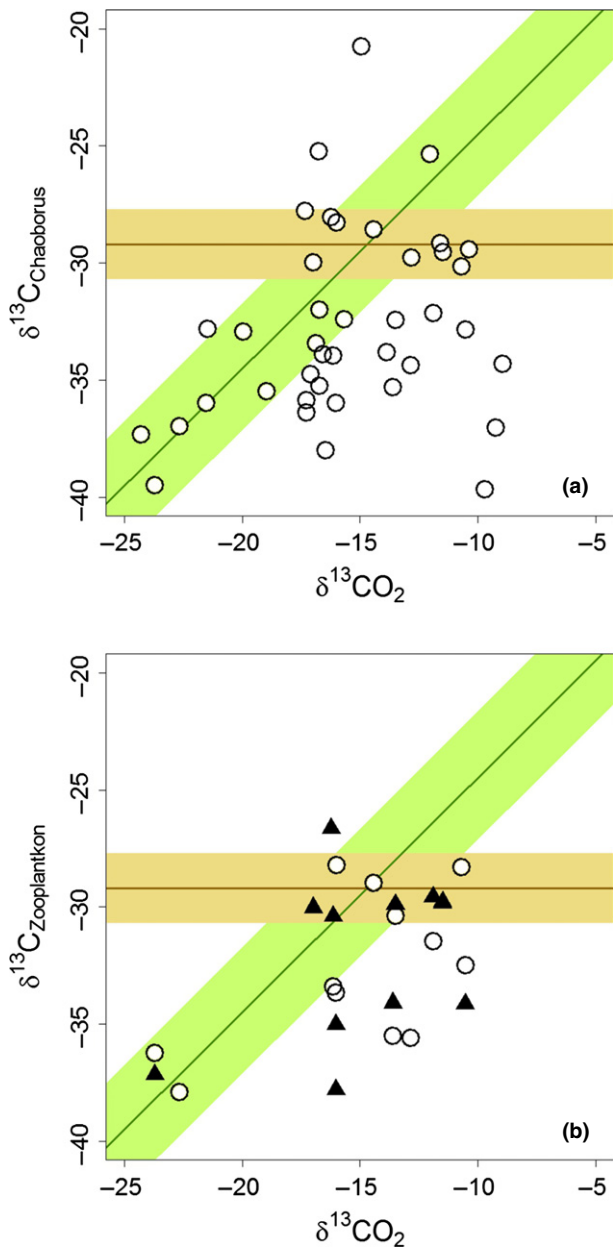
Fig. 2 (A) *Chaoborus* $\delta^{13}C$ in the 40 lakes plotted with the allochthonous end member (line = mean, shaded region = SD of 81 samples, allochthonous $\delta^{13}C$ not influenced by $\delta^{13}CO_2$ in the lake) and algal end member (line = mean ε_C, shaded region = ε_C SD, phytoplankton $\delta^{13}C$ is dependent on the $\delta^{13}CO_2$ in the lake; see Equation 2). (B) Cladoceran (white circles) and copepod (black triangles) $\delta^{13}C$ with end members (same as above).
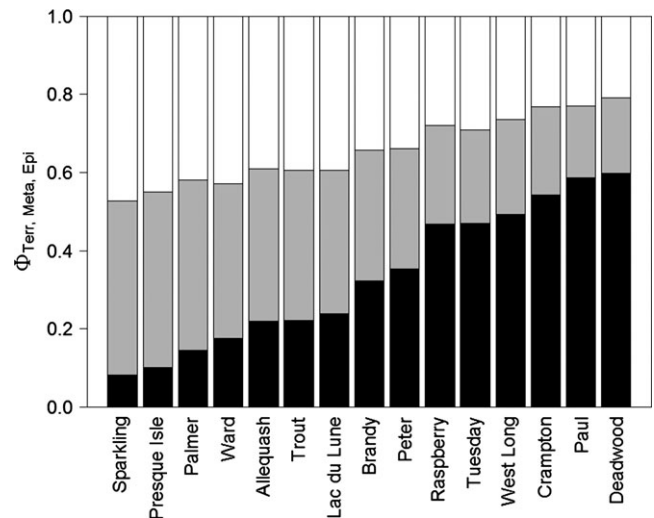

Fig. 3 Median value of the posterior distributions of the three-source, two-isotope Bayesian mixing model for the 15 lakes with an identified metalimnion resource pool. Lakes are ordered from lowest to highest consumer allochthony. *Chaoborus* ϕ_{Terr} in each lake is the black portion of the bar, ϕ_{Meta} is the grey portion of the bar, and ϕ_{Epi} is the white portion of the bar.

included lake surface area (SA) as the independent variable ($R^2 = 0.49$; Figure 4). The next highest ranked model ($R^2 = 0.55$) included a combination of lake surface area and DIC concentration. There were no significant regression models for copepod ϕ_{Terr} and any independent variable. There was one regression model for cladoceran ϕ_{Terr} with the concentration of chlorophyll α

([Chla]) as the independent variable ($y = 0.01 + 0.03*$ [Chla]; $R^2 = 0.54$).

Chaoborus ϕ_{Terr} was significantly related to POM ϕ_{Terr} (Figure 5; $R^2 = 0.4$) but the median *Chaoborus* ϕ_{Terr} was generally less than POM ϕ_{Terr} in the same lakes. This difference is consistent, with the slope of the POM ϕ_{Terr} versus *Chaoborus* ϕ_{Terr} relationship, which was significantly <1 (slope = 0.55, SE = 0.1). Cladoceran and copepod ϕ_{Terr} were not significantly correlated with POM ϕ_{Terr}.

Discussion

Allochthony of pelagic consumers

Based on the graphical analysis of the *Chaoborus* and zooplankton δ^2H data, there was a large range in the reliance of pelagic consumers on allochthonous resources in the sampled lakes. The lack of a relationship between consumer δ^2H and δ^2H_2O indicates that *Chaoborus* biomass as a whole among lakes was not dominated by either allochthonous or autochthonous resources, but rather was a variable mixture of these sources. *Chaoborus* and zooplankton $\delta^{13}C$ values are reflective of the variability of δ^2H values, but not all of the values lie between the end members, yielding impossible estimates of consumer allochthony if $\delta^{13}C$ alone had been used for the analysis. Although the $\delta^{13}C$

Table 1 Results of the multiple linear regression analysis with $y = Chaoborus\ \phi_{Terr}$

Ind Var. 1	Ind Var. 2	Adj. R^2	AICc	Equation
SA	—	0.49	−35.43	$y = 0.52 − 0.06*log(SA)$
SA	DIC	0.55	−35.30	$y = 0.74 − 0.05*log(SA) - 0.05*log(DIC)$
DIC	Colour:TP	0.54	−34.82	$y = 0.60 − 0.05*log(DIC) + 0.08*log(colour:TP)$
SA	POM ϕ_{Terr}	0.54	−34.10	$y = 0.33 − 0.04*log(SA) + 0.25* POM\ \phi_{Terr}$
Colour:TP	–	0.46	−33.20	$y = 0.32 + 0.1*log(colour: TP)$
Colour	DIC	0.52	−33.15	$y = 0.71 + 0.07*log(colour) − 0.08*log(DIC)$
Colour	TP	0.52	−33.04	$y = 0.35 + 0.1*log(colour) − 0.22*log(TP)$
Colour:Chla	DIC	0.51	−32.70	$y = 0.75 + 0.08*log(colour:Chla) − 0.06*log(DIC)$
SA	TP	0.52	−32.35	$y = 0.54 − 0.06*log(SA) − 0.09*log(TP)$
CA	–	0.44	−32.04	$y = 0.58 − 0.05*log(CA)$
SA	Colour:Chla	0.51	−31.94	$y = 0.51 − 0.04*log(SA) + 0.04*log(colour:Chla)$
SA	Colour:TP	0.51	−31.86	$y = 0.43 − 0.04*log(SA) + 0.05*log(colour:TP)$
POM ϕ_{Terr}	Colour:TP	0.51	−31.82	$y = 0.18 + 0.27*POM\ \phi_{Terr} + 0.07*log(colour:TP)$
CA	POM ϕ_{Terr}	0.51	−31.67	$y = 0.34 − 0.03*log(CA) + 0.28*POM\ \phi_{Terr}$

SA, lake surface area (ha); DIC, dissolved inorganic carbon (μM); TP, total phosphorus (μg L$^{−1}$); CA, catchment area (ha).
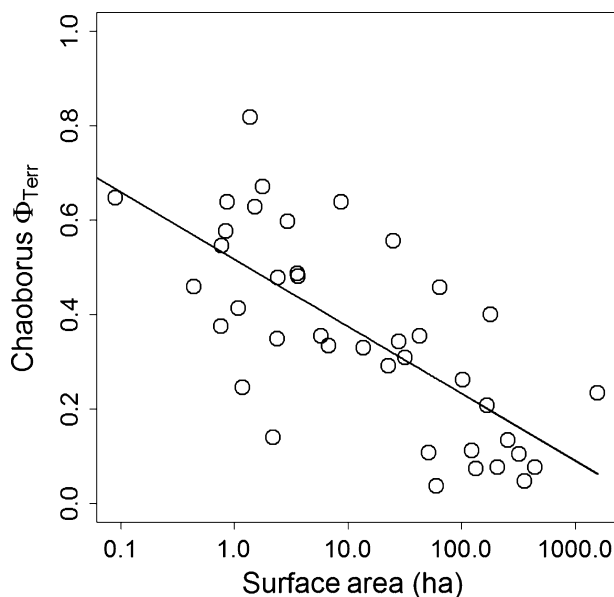

Fig. 4 The relationship between lake surface area and *Chaoborus* ϕ_{Terr} [$y = 0.52–0.06*log(x)$; $R^2 = 0.49$; *P*-value < 0.01].

Fig. 5 The linear regression model of allochthonous resource availability (POM ϕ_{Terr}) and consumer utilisation (*Chaoborus* ϕ_{Terr}). The dashed line is the 1 : 1 line, and the solid line is the least squares regression ($R^2 = 0.45$).

values did not align with the regional end members, the variability of consumer $\delta^{13}C$ values among lakes was similar to that of consumer δ^2H values. It is possible that a more depleted source, such as methanotrophic bacteria, was unaccounted for. Consumer $\delta^{13}C$ is not subject to large trophic discrimination (Vander Zanden & Rasmussen, 2001; Post, 2002). Therefore, the similar pattern of variability among lakes between the uncorrected consumer $\delta^{13}C$ and the δ^2H values provides support for the variability in allochthonous support of consumers modelled using the δ^2H values alone.

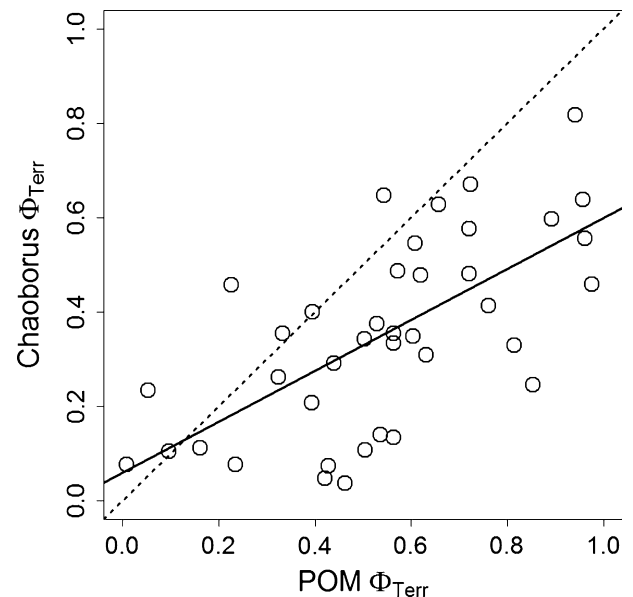The range of consumer allochthony reported here based on the two-source model is representative of prior allochthony estimates for pelagic consumers of <5% allochthonous in clear-water systems (Pace *et al.*, 2007; Francis *et al.*, 2011) to >75% allochthonous in humic systems (Carpenter *et al.*, 2005; Kankaala *et al.*, 2010; Karlsson *et al.*, 2012). Additionally, although these were point estimates of allochthony, five of the lakes included in this study have been used in previous seasonal allochthony studies. The median point estimates in this study are all within 10% of the median estimates (well within the estimate error) of these longer-term studies (Carpenter *et al.*, 2005; Solomon *et al.*, 2011; Batt *et al.*, 2012).

The allochthony of zooplankton in the subset of 18 lakes was variable (linear regressions non-significant) and represented a broad gradient of resource use. On average, the allochthony of cladoceran consumers was higher than the allochthony of copepod consumers among systems. This pattern and average proportion of allochthonous support are consistent with the patterns observed in other studies (Pace *et al.*, 2007; Solomon *et al.*, 2011; Berggren, *in review*). This pattern is supported by the evidence that cladocerans directly graze on bacteria that are utilising allochthonous DOM (Karlsson *et al.*, 2004; Taipale *et al.*, 2008), while copepods are more selective for autochthonous resources (Pulido-Villena *et al.*, 2005). The significant effect of zooplankton feeding strategy on the use of allochthonous resources observed here and in other studies highlights the utility of sampling an integrating consumer such as *Chaoborus* to compare allochthonous resource use among systems.

Importance of deep phytoplankton resources to consumers

Chaoborus ϕ_{Terr} was not significantly different when modelled using two sources (phytoplankton and allochthonous material) versus three sources (epilimnetic and metalimnetic phytoplankton and allochthonous resources). Francis *et al.* (2011) concluded that metalimnetic phytoplankton, and not terrestrial organic matter, was the isotopically depleted resource consumed by zooplankton in their study systems. From our analysis, we cannot conclude that epilimnetic and metalimnetic phytoplankton alone contribute to zooplankton biomass. Instead, when metalimnetic phytoplankton was included as a potential resource, the allochthonous fraction of consumers did not change significantly, and only the epilimnetic fraction was decreased. Our findings are consistent with other studies that have found concurrent use of epilimnetic, metalimnetic and terrestrial resources by migrating zooplankton consumers (Matthews & Mazumder, 2006; Batt *et al.*, 2012). Based on the findings of Francis *et al.* (2011) and Batt *et al.* (2012), we might expect metalimnetic resources to be more important to consumers in deep, clear-water lakes and naturally productive systems. However, due to the uncertainty in the model and the small difference in autochthonous isotope resource values, distinguishing metalimnetic resource use is not possible for the set of lakes we studied using ambient isotopes of H and C.

Distinguishing the epilimnion and metalimnion end members requires a large difference in the isotope value of the water or CO_2 between the layers. The mean difference in δ^2H_2O between the epilimnion and metalimnion of the 15 lakes with an oxygen maximum was 2.8 ‰ (±2.1 SD), and the mean difference in $\delta^{13}CO_2$ was 2.7 ‰ (±3.7 SD). Neither the difference in δ^2H_2O or $\delta^{13}CO_2$ is large enough to discriminate epilimnetic and metalimnetic sources given the variability surrounding the phytoplankton end member (derived from ε_H and ε_C). Additionally, variability in ε_C among systems has been demonstrated numerous times in the literature (e.g. Bade *et al.*, 2006; Gu *et al.*, 2011) as also has taxonomic variability in phytoplankton $\delta^{13}C$ (Vuorio, Meili & Sarvala, 2006). The variance of the ε_C estimate alone was sufficiently large to exceed the small isotopic difference between phytoplankton and terrestrial $\delta^{13}C$ end members. To fully examine the importance of the metalimnetic resource pool, separation of the two autochthonous resources through isotope enrichment or some other tracer would be useful.

The goal of Bayesian isotope analyses such as ours is to incorporate as many of the uncertainties of stable isotope analyses as possible (Fry, 2013). Perhaps the largest uncertainty is the δ^2H isotope value of the phytoplankton end member, which we have estimated using all measurements of ε_H available in the region and applying those values across all lakes. Although this method appears to be robust because the ε_H values used in our study are very similar to the values from other regions (Caraco *et al.*, 2010; Berggren *et al. in review*), further investigation is warranted to determine the variability of ε_H as well as environmental factors that may impact ε_H systematically. Additional uncertainty associated with the δ^2H isotope approach derives from the proportion of direct water use in the diet, trophic fractionation, differences between bulk organic matter and specific biochemical pools (e.g. lipids) and technical issues associated with measurement (e.g. exchangeable hydrogen). We estimated dietary water from our data, so this aspect of uncertainty is accounted for in the model. Furthermore, we have observed relatively consistent values for field populations using different approaches to estimate this parameter (Solomon *et al.*, 2009). Trophic fractionation is small (Solomon *et al.*, 2009). We measured only small differences when we compared various whole-tissue samples to lipid-extracted samples (J.J. Cole, unpublished data). The problem of exchangeable hydrogen for most samples appears small (Chesson *et al.*, 2009), and this finding is supported by the relative consistency of an algal standard used in the analyses. Hence, the δ^2H approach appears robust to a variety of possible problems, especially given large differences in the end members. Nevertheless, additional analysis of the method and associated uncertainties is warranted.

In our analysis, we did not consider the possibility that pelagic consumers use methanotrophic bacteria (Jones & Grey, 2011). Prior consideration of methane as a possible source in a few of the sampled lakes (Pace *et al.*, 2007; Cole *et al.*, 2011) indicated that organic production derived from methane was unlikely to make a large contribution to pelagic consumers. In addition, the *Chaoborus* and zooplankton δ^2H data fell neatly within the chosen end member distributions, and therefore, a more complex model did not seem warranted. Nevertheless, methane can be a significant source of carbon, especially for benthic consumers (Deines, Wooller & Grey, 2009), and we cannot rule out that direct or indirect consumption of methane-oxidising bacteria influenced the isotope patterns we observed, particularly the relatively depleted carbon-13 of *Chaoborus* and zooplankton.

Patterns of consumer allochthony

Consumer allochthony is highly variable among ecosystems, and therefore, the notion that a particular consumer is allochthonous or not is an oversimplification. The covariates that were chosen in the regression analyses are all likely surrogates for lake carbon sources. The goodness of fit of these models is similar, and explanatory covariates were not strongly discriminated by the data. Using an AIC_C approach, the regression model selected as the best included only lake surface area. Decreasing consumer allochthony with increasing lake size is a pattern that has been previously observed for lakes in this area (Pace *et al.*, 2007) and in other regions (Zigah *et al.*, 2012) and consumers (Babler, Pilanti & Vanni, 2011). Given that the global distribution of lake abundance is dominated by small lakes (Downing *et al.*, 2006) and given the surface area relationship observed in this study and in others, it is likely that allochthonous inputs are important for aquatic consumers in a large number of systems. This is also consistent with the global evidence that unproductive lakes are heterotrophic due to the metabolism of allochthonous organic carbon (Sobek, Tranvik & Cole, 2005), some of which is supporting secondary production.

Lake size is also negatively correlated with resource ratio variables such as the colour/chlorophyll ratio and, similarly, the colour/TP ratio. The ranges in lake size and in colour/chlorophyll ratio that were available when this hypothesis was originally posed (Pace *et al.*, 2007; Batt *et al.*, 2012) only represent a small fraction of the range observed in this study. From the relationships in our study, we can confirm the pattern previously observed; allochthony is lower in large, clear-water lakes and higher in smaller, more highly coloured lakes. Additionally, allochthony is lower in eutrophic lakes and higher in dystrophic lakes.

There is clearly a relationship between resource availability and utilisation by pelagic consumers. The linear regression model of POM ϕ_{Terr} versus *Chaoborus* ϕ_{Terr} explains over 40% of the variation in consumer allochthony among systems, which is consistent with other linear models of this relationship from other studies (Marcarelli *et al.*, 2011). The model predicts consumer ϕ_{Terr} to be below the 1 : 1 line, indicating some degree of preferential assimilation of autochthonous resources. The preferential use of autochthonous resources probably reflects the higher food quality of algae to the zooplankton upon which *Chaoborus* preys (Marcarelli *et al.*, 2011). Perhaps most importantly, the model has a non-zero slope (*P*-value <0.01), indicating that although there is preferential feeding on autochthonous resources, allochthonous resources are important to consumers across a large gradient of inputs and lake types.

Considering that the data are point estimates of both POM and *Chaoborus* allochthony during the season, the differing turnover rates of the carbon pools, and that *Chaoborus* diets include a range of items from lower trophic levels, the explanatory power of the linear regression models is reasonably high. Some of the additional variability could be due to differences in food-web structure that cause differences in consumer behaviour among lakes with similar resource availability (von Ende, 1979) or seasonality in resource use (Taipale *et al.*, 2009; Rautio, Mariash & Forsström, 2011). Additionally, these models do not incorporate any measure of resource quality or characterise the pathway by which allochthonous material entered the POM pool, which could help explain the variation in allochthonous use among systems with similar resource availability ratios (Karlsson *et al.*, 2003; Marcarelli *et al.*, 2011).

The results from this survey support the hypothesis that pelagic zooplankton in some lakes obtain some of their organic material from the catchment. The point estimates of allochthony for any one lake are uncertain for a variety of reasons, including unknown or unmeasured food sources and uncertainty in values such as photosynthetic fractionation or dietary water (Soto, Wassenaar & Hobson, 2013). However, the pattern of estimates of allochthony among lakes seems reasonable. In clear-water and eutrophic lakes, we estimated low values of allochthony; in small, highly coloured lakes, we estimated higher values. Our findings illustrate the variability in connectivity between aquatic consumers and the surrounding terrestrial catchment. We have

demonstrated that both the range among systems and degree of consumer allochthony within systems are substantial. We have also characterised the relationship between allochthonous resource availability and utilisation, something that was previously poorly defined due to a lack of consumer allochthony measurements in a diversity of systems. The overarching patterns indicate that allochthonous resources contribute to food webs in many non-eutrophic lakes. With the exception of a few studies (Loreau & Holt, 2004; Rubbo *et al.*, 2012), the influence of allochthonous relative to autochthonous resource use in food-web interactions, dynamics and stability remains a largely unexplored but worthy subject.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Computation of the Source Proportions.

Table S1. The isotope values (‰) of the water, dissolved carbon dioxide and *Chaoborus* for the 40 survey lakes. The second portion of the table presents isotope values for selected zooplankton taxa.

(Manuscript accepted 23 May 2013)